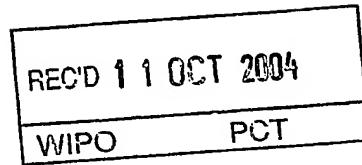




PCT / SE 2004 / 001369

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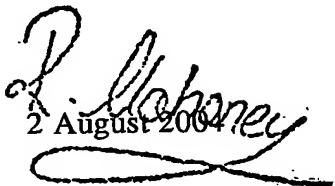
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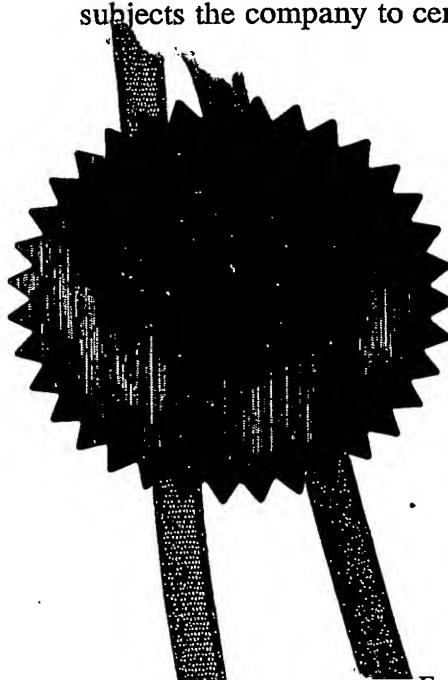
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2 August 2004



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1. Your reference

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89 77879/001

0322645.3

2. Patent application number
(The Patent Office will fill in this part)3. Full name, address and postcode of the
or of each applicant (underline all surnames)Melacure Therapeutics AB
Ulleråkersvägen 38
SE-756 43 Uppsala
Sweden

Patents ADP number (if you know it)

08566010001

29SEP03 E840394-17 000027
P01/7700 0.00-0322645.3If the applicant is a corporate body, give
country/state of its incorporation

SE

4. Title of the invention

Use of antisecretory factor peptides

5. Name of your agent (if you have one)

Frank B. Dehn & Co.

"Address for service" in the United Kingdom
to which all correspondence should be sent
(including the postcode)179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number (if you know it)

166001✓

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Priority application number
(if you know it)Date of filing
(day / month / year)7. Divisionals, etc: Complete this section only if
this application is a divisional application or
resulted from an entitlement dispute (see note f)

Number of earlier UK application

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Answer YES if:

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Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	0
Description	18
Claim(s)	8
Abstract	1
Drawing(s)	6 + 6

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for a preliminary examination and search (Patents Form 9/77)

Request for a substantive examination (Patents Form 10/77)

Any other documents (please specify)

1

1 page of separate listing

11.

I/We request the grant of a patent on the basis of this application.

Signature

Date 26 September 2003

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Philip Webber
01273 244200

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USE OF ANTI-SECRETORY FACTOR PEPTIDES

The present invention relates to the use of polypeptides comprising certain fragments of anti-secretory factor (AF) in the manufacture of a medicament for treating and/or preventing a condition that is characterised by or associated with the pathological loss and/or gain of cells, such as the pathological degeneration, loss of regeneration ability and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell. Medical conditions characterised by or associated with such conditions include damage to the brain by trauma, asphyxia, toxins, hypoxia, ischemia, infections or degenerative or metabolic insults resulting in neurological and/or cognitive defects.

Traumatic, asphyxial, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS) often result in damage to several different cell types. As an example of a degenerative insult to the CNS, Parkinson's disease often causes loss of specific populations of cells and is in particular associated with the specific loss of dopaminergic neurons in the Substantia nigra. Similarly, multiple sclerosis is associated with loss of myelin and oligodendrocytes. Another illustration of a degenerative disorder caused by selective loss of a specialised type of neurons is Alzheimer's disease, which is associated with loss of cholinergic neurons. Additionally, there are many other instances in which CNS injuries or diseases can cause damage to oligodendroglia, astroglia, or neuronal cells.

In general, replacement of neurons following degeneration or damage is not a characteristic of the adult mammalian brain. Neuronal loss is thus usually considered permanent. Nonetheless, prolonged postnatal neurogenesis has been described in the granule cell layer of the hippocampal formation to persist well into adulthood in several species, including man (Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A., Nordborg, C., Peterson, D.A., and Gage, F.H., *Nature Med.* 11: 1313-1317 (1998)). This phenomenon is attributed to the existence of an omnipotent neuronal cell population, the neuronal progenitor cells. Neuronal progenitor cells are stem cells and reside in the subgranular zone (SGZ) of the dentate gyrus where they continuously proliferate, migrate into the granule cell layer and differentiate into granule cells. The

new-born neurons in the granule cell layer express markers of differentiated neurons and have morphological characteristics corresponding to differentiated granule cells. Furthermore, they establish axonal processes into the mossy fibre pathway and form synaptic connections with their targets in hippocampal layer CA3 (Gage, F.H. *Science* 287:1433-1438 (2000)). The neurogenesis in the dentate gyrus is in itself especially intriguing as the hippocampal formation in the dentate gyrus is intimately associated with spatial learning and memory (McNamara, R.K. and Skelton, R.W., *Brain Res. Rev.* 18: 33-49 (1993)). It has previously been shown that the proliferation of progenitor cells in the SGZ can be influenced by the administration of n-methyl-d-aspartate (NMDA) receptor antagonists or by the removal of the adrenal glands (Cameron, H.A. and Gould, E., *Neuroscience* 61: 203-209 (1994); Cameron, H.A., Tanapat, P. and Gould, E., *Neuroscience* 82: 349-354 (1998)). What is more, it has been shown that exposure to enriched environments leads to an increased number of surviving, newly formed granule cells as well as to an increased total number of neurons in the dentate gyrus (Kempermann, G., Kuhn, H.G. and Gage, F.H., *Nature* 386: 493-495 (1997)).

In addition to the potential of replacing neurons following degeneration or damage, neuronal progenitor cells are also thought to be important for the ability to learn. In accordance with the fact that neuronal plasticity is reduced with increased age, recent studies have demonstrated that proliferation of progenitor cells is also decreased (Kuhn, H., Dickinson-Anson, H., and Gage, F.H., *J. Neurosci.* 16: 2027-2033 (1996)).

Interestingly, stem cells that were isolated from the adult rodent brain have been transplanted into the hippocampus of adult animals where they differentiated into cells with neuronal characteristics (Suhonen, J.O., Peterson, D.A., Ray, J., and Gage, F.H., *Nature* 383:624-627 (1996)). Moreover, stem cells from one germinal layer were in addition seen to be able to form tissue from a different germinal layer (Anderson DJ, Gage FH, Weissman IL. *Nat Med.* 4:393-395 (2001)).

Stem cells have successfully been isolated from different tissue sources (Gage F.H. *Science* 287:1433-1438 (2000)) and even human embryonic stem cell lines (ES -cells) have been established from pre-implantation embryos (Thomson et al., *Science* 282: 1145-1147 (1998)). These ES-cells were shown to maintain a stable developmental potential to form advanced derivatives of all three embryonic germ layers. As is easily understood, human ES cell lines and stem cell lines derived from different tissues like

skin, blood or brain have widespread applications for human developmental biology, drug discovery, drug testing and transplantation medicine.

5 Unfortunately, in order to facilitate autologous transplantation of neuronal cells, it is still necessary to use adult-derived cells which are complex to isolate and to propagate.

Anti-secretory factor (AF) is a polypeptide which occurs naturally in human body fluids (Bjorck, S., Bosaeus, I., Ek, E., Jennische, E., Lonnroth, I., Johansson, E., and Lange S., Gut 46(6):824-829 (2000)). AF known to inhibit intestinal fluid secretion induced by cholera toxin (Johansson, E., Lonnroth, I., Lange, S., Jonson, I., Jennische, E., and Lonnroth, C., J. Biol. Chem. 270(35):20615-20620 (1995)) and it has also been shown to suppress intestinal inflammation and hypersecretion in rat induced by toxin A from Clostridium difficile (Johansson, E., Jennische, E., Lange, S., and Lonnroth, I., Gut 41(5) 719-720 (1997)). AF is a 41 kDa protein consisting of 382 amino acids. It appears as a 60 kDa protein on SDS-polyacrylamide gels (Johansson, E., Lonnroth, I., Lange, S., Jonson, I., Jennische, E., and Lonnroth, C., J. Biol. Chem. 270(35):20615-20620 (1995)). The active site has been located in a small region of the AF-sequence, between amino acids 36 and 42 (Johansson, E., Lange, S., and Lonnroth, I., Biochim. Biophys. Acta 1362 (2-3):177-182) 1997). Anti-secretory factor peptides regulating pathological permeability changes are described in WO 97/08202. However, no effect on proliferation or differentiation in any cell type is disclosed in the aforementioned publication.

25 The present inventors have now surprisingly found that certain fragments of AF are capable of inducing proliferation of progenitor cells derived from the adult CNS. This suggests a new and exiting mode of action involving AF fragments in mediating the survival, proliferation and/or propagation of these cells in vitro as well as in vivo. In particular, the inventors have found that certain AF fragments can modulate the proliferation, differentiation, migration and/or programmed cell death of a neural stem cell or progenitor cell from the adult CNS. The present invention thus provides new and improved means to treat injuries to or diseases or disorders of the central or peripheral nervous system, inter alia, and provides possibilities for influencing the neuronal plasticity of the CNS.

35 The mammalian brain, including the human brain, retains its ability to generate neurons throughout life in certain regions only. New neurons and astroglial cells and oligodendrocytes are generated by cell genesis from stem or progenitor cells. During

the research leading to the present invention, it was found that certain AF fragments induce an increase in cell genesis from progenitors and/or stem cells *in vitro* as well as in the adult brain.

5 It has now been found that it is possible to treat neural loss suffered after a CNS insult or in the progress of a neuronal disease or disorder, by increasing the number of stem cell or progenitor derived cells, including neurons, astroglial cells and/or oligodendrocytes, by administering an effective amount of certain AF fragments. The concentration of the AF fragment may be increased in the CNS of the patient with the

10 intention of inducing proliferation and/or differentiation of stem cells or progenitor cells with a concomitant increase in cell genesis. It is thus possible to effect cell genesis from stem cells or progenitor cells and thus induce the genesis of neurons and/or glial cells after either neuronal, oligodendroglial or glial cell loss in the CNS or PNS, or to prevent the normal age-related deterioration of said cells in the CNS or PNS.

15

One embodiment of the invention relates to the use of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

20

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent

X2 is H, R or K

X3 is S or L

25

X4 is T or A

X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof,

in the manufacture of a medicament for the treatment and/or prevention of a condition associated with or characterised by a pathological loss and/or gain of cells.

30

Preferably, Formula I has the sequence of one of:

35

- a) amino acids nos. 35-42 of SEQ ID NO:1
- b) amino acids nos. 35-46 of SEQ ID NO:1
- c) amino acids nos. 36-51 of SEQ ID NO:1
- d) amino acids nos. 36-80 of SEQ ID NO:1
- e) amino acids nos. 1-80 of SEQ ID NO:1,

or a pharmaceutically acceptable salt thereof.

SEQ ID NO:1 is the first part of the amino acid sequence of the anti -secretory factor polypeptide as given in Johansson, E., et al., Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. J Biol. Chem., 1995. 270(35): p. 20615-20; WO9708202 and US 6,344,440.

The invention also relates to uses of pharmaceutically acceptable salts of the polypeptides comprising an amino acid sequence of Formula I.

10 The invention also provides a method of treating and/or preventing a condition associated with or characterised by a pathological loss and/or gain of cells, comprising the administration to a patient of an effective amount of a polypeptide comprising an amino acid sequence of Formula I.

15 The invention also provides a polypeptide comprising the amino acid sequence of Formula I for use in treating and/or preventing a condition associated with or characterised by a pathological loss and/or gain of cells.

20 In some embodiments of the invention, the polypeptides of Formula I may additionally comprise terminal protecting groups. Examples of N-terminal protecting groups include acetyl. Examples of C-terminal protecting groups include amide.

25 The term "pathological loss and/or gain of cells" is in the present context used to describe the common technical feature of a number of medical conditions and disorders. These conditions and disorders are characterised by displaying pathological degeneration of, loss of ability of regeneration of and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

30 The condition to be treated may be caused, inter alia, by one or more of traumatic, asphyxia, neuropathic pain, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS). These often result in damage to several different cell types. Thus, damage to the brain by trauma, asphyxia, toxins, 35 ischemia or infections frequently causes neurological and/or cognitive defects. In other cases, the condition may be caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder. In yet other cases, the condition may be caused by

genetic factors or the cause may be unknown. In yet further cases, the condition may be caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, and/or damage to the spinal cord after disease and/or trauma.

5

In one embodiment of the invention, the condition to be treated is a condition that is associated with or characterised by a pathological loss and/or gain of cells in the peripheral nervous system (PNS) or central nervous system (CNS).

- 10 Cells that may be affected by a polypeptide comprising an amino acid sequence of Formula I are, for example, embryonic stem cells, adult stem cells, progenitor cells and/or cells derived from them. They can belong to any or all of the three germinal layers. Once stimulated, the above-listed stem cells will differentiate into and/or replace malfunctioning, dying, or lost cells or cell populations, such as in pathological
- 15 CNS or PNS conditions characterised by abnormal loss of cells, oligodendroglia, astroglia, and/or neuronal cell and/or cell population, such as non-cholinergic neuronal cell, cholinergic neuronal cell, and/or glial cell and/or cell populations.

20 The invention particularly relates therefore to the treatment of conditions associated with or characterised by a loss and/or gain of stem cells, preferably neural stem cells, for example adult or embryonic stem cells; or conditions associated with or characterised by a loss and/or gain of progenitor cells. In one embodiment, the stem cells are hippocampal stem cells, preferably adult-derived hippocampal stem cells.

25 The invention also relates particularly to the treatment of conditions associated with a loss and/or gain of differentiated cells. In one preferred embodiment, the differentiated cells are chondrocytes, cardiomyocytes, oligodendroglia, astroglia, neuronal cells, epithelial cells, endothelium, skin, blood, liver, kidney, bone, connective tissue, lung tissue, exocrine gland tissue and/or endocrine gland tissue or muscle cells. Preferably, 30 the differentiated cells are neural cells, neurons, astrocytes, oligodendrocytes, Schwann cells or glial cells. The neural cells may, inter alia, be non-cholinergic neuronal cells or cholinergic neuronal cells.

35 The invention also provides the use of a polypeptide comprising an amino acid sequence of Formula I in the manufacture of a medicament for modulating the development of stem cells and/or progenitor cells.

A further embodiment of the invention provides a method of modulating the development of stem cells and/or progenitor cells, comprising contacting the stem cells and/or progenitor cells in vivo or ex vivo with an amount of a polypeptide comprising an amino acid sequence of Formula I.

5

The invention further provides a polypeptide comprising an amino acid sequence of Formula I for use in modulating the development of stem cells and/or progenitor cells in vivo or ex vivo.

10 In the context of the present invention, the term "modulating the development of stem cells and/or progenitor cells" includes one or more of inducing proliferation, inducing programmed cell death, maintaining the genesis, promoting differentiation and promoting migration of stem cells and/or progenitor cells.

15 The uses and methods of the invention are preferably suited for the treatment of abnormal and/or medical conditions affecting pathological loss or gain of neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells. They may thus be used to prevent, treat or ameliorate damage, diseases or deficits of the CNS or the PNS. The pharmaceutically active substance used according to the invention is especially suitable for treatment of conditions affecting the oligodendroglia, astroglia, and/or neuronal cells. Such conditions may, for example, be due to CNS damage or deficits, neuronal cell loss or memory loss. Such conditions may be caused by a number of different factors or diseases, e.g. traumatic, malignant, inflammatory, auto-immune or degenerative disorders such as multiple sclerosis, 20 hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Meniere's disease and demyelition disorder. The effect of the pharmaceutically active substances used according to this preferred embodiment of the invention is due to their ability either to induce cell genesis, proliferation and/or differentiation of progenitor derived cells in or from the central nervous system.

25

Nonetheless, and as outlined above, the present invention is not restricted to uses and methods for treating neuronal diseases and conditions, but said uses and methods may also be used for treating a large variety of mammalian conditions that are characterised by pathological cell loss and/or gain, such as Parkinson's disease (dopaminergic neurons), Alzheimer's disease (cholinergic neurons), Multiple Sclerosis (oligodendrocytes), stroke (neurons and glial cells), asphyxia or hypoxia (neurons and glial cells), heart failure (cardiomyocytes), heart infarction (cardiomyocytes), diabetes

(pancreatic beta cells), artrosis or arthritis (chondrocytes), skin disease and burn injuries (dermis and epidermis), liver diseases or failure (hepatocytes), muscle diseases or damages (myocytes), cancer (tissues affected by cancer), pancreatic dysfunction (exocrine or endocrine pancreatic cells) and inflammatory bowel disease (intestinal cells).

5

The invention further relates to a pharmaceutical composition comprising a polypeptide comprising the amino acid sequence of Formula I for use in treating a condition associated with or characterised by a pathological loss and/or gain of cells.

10

Additionally, the invention provides a pharmaceutical composition comprising a polypeptide comprising an amino acid sequence of Formula I for use in modulating the development of stem cells and/or progenitor cells in vivo or ex vivo.

15

The pharmaceutical compositions or medicaments of the invention may additionally comprise one or more pharmacologically acceptable carriers, excipients or diluents, such as those known in the art.

20

The compositions or medicaments may be in form of, for example, fluid, semi-solid or solid compositions such as, for example but not limited to dissolved transfusion liquids, such as sterile saline, Ringer's solution, glucose solutions, phosphate buffered saline, blood, plasma or water, powders, microcapsules, microspheres, nanoparticles, sprays, aerosols, inhalation devices, solutions, dispersions, suspensions, emulsions and mixtures thereof.

25

The compositions or medicaments may be formulated according to conventional pharmaceutical practice, see, e.g., "Remington: The science and practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-3 and "Encyclopedia of Pharmaceutical Technology", edited by Swarbrick, J. & J. C. Boylan, Marcel Dekker, Inc., New York, 1988 ISBN 0-8247-2800-9.

30

The choice of pharmaceutically acceptable excipients in a composition or medicament for use according to the invention and the optimum concentration thereof may readily be determined by experimentation. Also whether a pharmaceutically acceptable excipient is suitable for use in a pharmaceutical composition is generally dependent on which kind of dosage form is chosen. However, a person skilled in the art of pharmaceutical formulation can find guidance in e.g., "Remington: The science and

35

practice of pharmacy" 20th ed. Mack Publishing, Easton PA, 2000 ISBN 0-912734-04-

3.

5 A pharmaceutically acceptable excipient is a substance, which is substantially harmless to the individual to which the composition will be administered. Such an excipient normally fulfils the requirements given by the national drug agencies. Official pharmacopoeias such as the United States of America Pharmacopoeia and the European Pharmacopoeia set standards for well-known pharmaceutically acceptable excipients.

10 The following is a review of relevant pharmaceutical compositions for use according to the invention. The review is based on the particular route of administration. However, it is appreciated that in those cases where a pharmaceutically acceptable excipient may be employed in different dosage forms or compositions, the application of a particular pharmaceutically acceptable excipient is not limited to a particular dosage form or of a 15 particular function of the excipient.

Parenteral compositions:

20 For systemic application, the compositions according to the invention may contain conventional non-toxic pharmaceutically acceptable carriers and excipients, including microspheres and liposomes.

25 The compositions for use according to the invention may include all kinds of solid, semi-solid and fluid compositions. Compositions of particular relevance are e.g. solutions, suspensions and emulsions.

30 The pharmaceutically acceptable excipients may include solvents, buffering agents, preservatives, chelating agents, antioxidants, stabilisers, emulsifying agents, suspending agents and/or diluents. Examples of the different agents are given below.

Examples of various agents:

Examples of solvents include but are not limited to water, alcohols, blood, plasma, spinal fluid, ascites fluid and lymph fluid.

Examples of buffering agents include but are not limited to citric acid, acetic acid, tartaric acid, lactic acid, hydrogenphosphoric acid, bicarbonates, phosphates, diethylamine, etc..

5 Examples of chelating agents include but are not limited to sodium EDTA and citric acid.

Examples of antioxidants include but are not limited to butylated hydroxy anisole (BHA), ascorbic acid and derivatives thereof, tocopherol and derivatives thereof,

10 cysteine, and mixtures thereof.

Examples of diluents and disintegrating agents include but are not limited to lactose, saccharose, emdex, calcium phosphates, calcium carbonate, calcium sulphate, mannitol, starches and microcrystalline cellulose.

15

Examples of binding agents include but are not limited to saccharose, sorbitol, gum acacia, sodium alginate, gelatine, starches, cellulose, sodium carboxymethylcellulose, methylcellulose, hydroxypropylcellulose, polyvinylpyrrolidone and polyetyleneglycol.

20 The pharmaceutical composition or the substance used according to the invention is preferably administered via intravenous peripheral infusion or via intramuscular or subcutaneous injection into the patient or via buccal, pulmonary, nasal or oral routes. Furthermore, it is also possible to administer the pharmaceutical composition or the pharmaceutically active substance through a surgically inserted shunt into a cerebral ventricle of the patient.

In one embodiment of the present invention, said pharmaceutical composition is formulated so that the active substance will pass into the ventricles of a patient's brain or into the cerebrospinal fluid of said patient, when it is administered to said patient.

30 This may e.g. be achieved by mechanical devices, vectors, liposomes, lipospheres, or biological or synthetical carriers.

Preferably, the administered dosage range is about 0.001-100 mg of a polypeptide comprising the amino acid sequence of Formula I per 100 g body weight, comprising a range of 0.001-100 mg/1g, 0.001-100 mg/10g and 0.001-100 mg/50g body weight. Preferably, the administered dosage range is about 0.001-100 mg of a polypeptide comprising the amino acid sequence of Formula I per 1kg body weight.

The invention may be used to treat humans or non-human mammals.

5 The terms "treatment" or "treating" as used herein relate to both therapeutic treatment in order to cure or alleviate a disease or a medical condition, characterised by abnormal loss and/or gain of cells, and to prophylactic treatment in order to prevent the development of a disease or a medical condition, characterised by pathological loss and/or gain of cells. Thus both prophylactic and therapeutic treatments are included in the scope of the present invention. The terms "treatment" or "treating" also refer to the 10 effecting of cell genesis from stem cells or progenitor cells by inducing the genesis of differentiated cells, such as e.g. neurons and/or glial cells after either neuronal, oligodendroglial or glial cell loss in the CNS or PNS, or to prevent the normal age-related deterioration in the CNS or PNS or other organs of the body. The treatment may either be performed in an acute or in a chronic way.

15 A polypeptide comprising the amino acid sequence of Formula I may also be used for the cultivation of stem cells or progenitor cells. The stem cells or progenitor cells may be used for the concomitant transplantation to a patient in need thereof, e.g. to the CNS, PNS, or other organs of the body, in said patient.

20 Thus the invention provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germlinal layer from a patient, characterised by:

25 a) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I to said patient prior to isolating said cell;
b) propagating said isolated cell *in vitro*; followed by
c) transplanting said propagated cells into the same or another patient in need thereof.

30 The invention also provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germlinal layer from a patient, characterised by:

35 a) isolating said cell and/or stem cell progeny from the patient;

b) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I to said isolated cell *in vitro* and propagating said cells; followed by
5 c) transplanting said propagated cells back into the same or another patient in need thereof.

The isolated cells of the above methods may be derived, *inter alia*, from the CNS and/or PNS of the patient.

10 AF-expanded stem and/or progenitor cells may be propagated and either pre-differentiated prior to grafting or allowed to differentiate as a result of interactions between the transplanted cells and the host. AF-expanded stem cells and/or progenitor cells may either be administered and/or grafted at a single time, or delivered repeatedly over a prolonged period. This will be especially useful if stem cells and/or progenitor 15 cells are administered to enter the target organ via the bloodstream.

According to another preferred embodiment of the invention, it is possible to use a polypeptide comprising the amino acid sequence of Formula I to propagate progenitor cells or stem cells or other cells in a tissue culture or a cell culture. Such cells may 20 thereafter be used for cell transplantation into a patient suffering from e.g. neuronal cell loss or a condition due to lack of endogenous cells of another type. The cells used to start the culture may either originate from the patient or from another human or animal donor, and may be used in the treatment of a broad variety of diseases and disorders comprising heart diseases such as infarct, diabetes, or in an assortment of neurological 25 diseases and disorders, such as those referred to above.

Thus the invention also provides a method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny *in vitro*, characterised by treating the isolated cell with a polypeptide comprising the amino acid 30 sequence of Formula I. Preferably, the isolated cell is selected from the group comprising chondrocytes, myocardial cells, blood cells, neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from said cells. In general, the isolated cell will be treated under appropriate conditions and for a time which is sufficient to achieve the desired propagation, induction, reduction and/or 35 maintenance.

When cells are to be removed from a patient for in vitro propagation, it may be advantageous first to increase the number of progenitor cells in the patient. This will better facilitate the subsequent isolation of said cells from patients. The number of progenitor cells are increased by use of the method or pharmaceutical composition 5 according to the invention.

A polypeptide comprising the amino acid sequence of Formula I, may be used alone or conjunction with other medicaments or growth factors such as epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2) or IGF -I, designed to induce cell genesis or 10 proliferation e.g. in the CNS or PNS. A polypeptide comprising the amino acid sequence of Formula I, alone or in conjunction with other medicaments, peptides, growth-factors, steroids, lipids, glycosylated proteins or peptides, used either simultaneously or in sequence, may be used in order to facilitate cell genesis or the generation of specific cell types in vivo or in vitro. It may also be used to induce 15 immature or multipotent cells to activate specific developmental programs as well as specific genes in the aforementioned cells.

By the above mentioned term "cell genesis" is meant the generation of new cells such as neurons, oligodendrocytes, schwan cells and astroglial cells from multipotent cells, 20 progenitor cells or stem cells within the adult CNS or PNS or other organs of the body, in situ or in isolation.

Furthermore, the invention also relates to the therapeutic use of substances that decrease the amount of active AF or naturally occurring analogous of AF in the patient 25 and thus decrease the genesis of new cells, e.g. of oligodendrocytes in patients with axonal or spinal cord injury, such as axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, and/or damage to the spinal cord after disease and/or trauma. Examples of such substances are drugs, antibodies, compounds, peptides and/or inhibitor of endogenous 30 AF release.

Since AF supports the genesis of new cells and especially neurons in the hippocampus, a structure intimately coupled to learning and memory, a polypeptide comprising the amino acid sequence of Formula I may be used in order to facilitate learning and 35 memory by the genesis of said cells.

Whilst the present invention relates primarily to a method for treating abnormal conditions in the CNS or PNS that are characterised by pathological loss and/or gain of cells, by affecting neural stem cells or progenitor cells, the uses and methods of the invention may be equally useful for treating and/or preventing medical conditions in 5 other organs of the body, provided that said medical conditions characterised by pathological loss and/or gain of cells.

Whilst reference has primarily been made herein to the use of polypeptides comprising an amino acid sequence of Formula I, the invention relates, mutatis mutandis, also to 10 polypeptides consisting essentially of an amino acid sequence of Formula I, and to polypeptides consisting of an amino acid sequence of Formula I.

The polypeptides comprising an amino acid sequence of Formula I may be produced by standard means, including recombinant and synthetic routes. 15

The invention will be more fully understood when reading the following Examples which are intended merely to illustrate, but not to limit, the scope of the invention.

FIGURE LEGENDS

20 **Figure 1**
To study the effect of AF-peptide on proliferation in adult derived hippocampal stem cells both the proportion of cells in mitosis (A) and the total number of cells (B) were counted. Cells in mitosis were positively stained by an antiserum recognising 25 phosphorylated histone H3 while total number of cells were counted using the nuclear dye staining Hoechst 33258. (** P < 0.001, ** P < 0.01, P < 0.05).

Figure 2

30 Illustrates proliferation measured as DNA content in adult derived hippocampal stem cell cultures after incubation with different concentrations of AF compared with untreated control cultures (red line) and cultures treated with 20ng/ml of bFGF (well known inducer of proliferation in stem cells). (* P < 0.05).

Figure 3

35 Complete nucleotide sequence of AF taken from Johansson, E., et al., Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion.

J Biol. Chem., 1995, 270(35): p. 20615-20, WO9708202, US6344440. Only the first part of the corresponding amino acid sequence is given.

5 EXAMPLES

Example 1: Proliferation experiments

For proliferation experiments, adult derived progenitor cells were passaged into polyornithine/laminin coated glass chamber slides (Nunc) at a density of approximately 0.7 x 10⁴ cells per cm². The cells were incubated for 48 hours in Dulbecco's Modified Eagles Medium / Hams's F12 (DMEM / F12, 1:1) containing N2 supplement (Life Sciences, Täby, Sweden) and 20 ng/ml recombinant human FGF-2 (Pepro Tech, IHC). The medium was then replaced with N2 medium without FGF-2 for another 48 hours.

10 Thereafter the medium was changed to N2 medium where a polypeptide consisting of amino acids 36-51 of SEQ ID NO:1 was added at different concentrations (10, 1, 0.1 and 0.01 ng/ml, respectively) for 48 hours. The cells were washed once in PBS and fixed in 4%-paraformaldehyde for 10 minutes in +4°C and rinsed twice (10 min) with PBS. Cells were incubated with an anti-phospho-histone H3 antisera (1:400, Upstate Biotechnology, Lake Placid, NY). As secondary antisera we used FITC conjugated sheep anti-rabbit (1:200, Boehringer Mannheim). The nuclear dye bisBenzimide 1:100 from stock at 5 µg/ml (Hoechst 33258, Sigma Chemical Co.) were used. FGF-2 was added in parallel with AF for comparison with a known substance that induce proliferation. Cells were counted using a Nikon Microphot-FX fluorescens microscope using a 40 times (40x) ocular. Ten area units (a.u.) were chosen by moving the focus from one end to the other of the coverslip. Cells in one circle using 40x ocular were counted as one a.u. Results are shown in Fig. 1. Doubled-blinded counting were performed in Fig. 1. The values in the figure are expressed as the mean ± SEM. Statistical analysis between groups were done using factorial analyses of variance

15 followed by a Fisher's post-hoc test.

20

25

30

Example 2: Quantitation of DNA content

The quantification of DNA content was performed according to manufacturer's instruction (Molecular Probes, OR) using the Cyquant DNA assay. Adult derived hippocampal stem cells were cultured at 0.2 x 10⁴ cells/cm² in 24-hole plates coated with polyornithine and thereafter laminin. The cells were cultured in N2 medium plus

35

bFGF for two days followed by N2 without bFGF for two days. Thereafter the cells were incubated in N2 medium without bFGF with a polypeptide consisting of amino acids 36-51 of SEQ ID NO;1 for 48 h, washed once in PBS and stored in -80 C freezer. Cells were thawed and resuspended in lysis buffer containing EDTA (1 mM), and

5 DNase-free RNase (Sigma) was then added to a final concentration of 1 µg/ml for 1 h at room temperature. Cyquant dye was added for 5 min at room temperature and DNA concentration measured by fluorescence spectroscopy at 480 nm excitation and 520 nm emission wavelengths using a Tecan Genobios microplate reader. Statistical analysis between control and each group were using Student's t-test.

10

Example 3:

The therapeutic efficacy of candidate cell lines are tested using the unilateral 6-hydroxydopamine-(6-OHDA)-lesion rat model for PD. In each recipient 50,000-

15 300,000 cells of a differentiated dopaminergic phenotype is stereotactically transplanted into the striatum. The hosts are treated with daily i.p. injections of cyclosporin A (10 mg/kg), a treatment which has previously been shown to efficiently diminish problems of immune rejection of human intracerebral xenografts in rats. The cells are treated with the lipid peroxidation inhibitor Tirilazad mesylate which is
20 already used both in experimental and in clinical trials to enhance graft survival.

The experiment demonstrates that AF expanded stem/progenitor cells survive and are integrated appropriately into the host brain.

25 **Functional assessments: Behaviour**

Amphetamine-induced rotational behaviour in rodents with unilateral 6-OHDA lesion is probably the most reliable and sensitive indicator of graft survival and function. Rotation is monitored in rats with unilateral 6-OHDA lesions prior to grafting and every 3 weeks after grafting. In addition, the capacity of the grafts to reverse deficits
30 under non-drug-induced conditions is examined in the so-called "stepping test". This experiment demonstrates that AF expanded stem/progenitors can ameliorate symptoms in a rat model of Parkinson's disease.

Dopamine production and release

35 To assess the intracellular content of neurotransmitter, striatal tissue levels of dopamine and its major metabolites are monitored in post-mortem samples using HPLC in rats 6-12 weeks after grafting.

To monitor the capacity of the grafted neurons to release synthesised dopamine, assessment of extracellular dopamine is examined using the intracerebral microdialysis technique under basal and drug-evoked (KCl, amphetamine, and apomorphine) 5 conditions, in rats that have exhibited graft-induced behavioural recovery. The experiment show the ability of AF expanded stem/progenitor cells to produce dopamine over long periods.

Example 4

10 To test whether administration of a pharmaceutical substance comprising AF or a polypeptide comprising the amino acid sequence of Formula I can be used to facilitate learning and memory, two groups of rats are subjected to memory testing. One of the groups is treated with AF. Rats from both groups are tested four weeks after BrdU 15 injection during four consecutive days. The rats are tested in a water maze with a video-tracking system. The time to reach the platform (latency) and the length of the swimming path are monitored. The escape platform is hidden 1 cm below the surface of the water at a fixed position. The water is made opaque by adding dry milk powder to the water. The water temperature is kept constant at 22oC throughout the test. Each 20 animal will be tested in four trials each day. Each trial lasts 45 s. Animals that fail to find the hidden platform within 45 s are designated as having a 45-s latency and are put on the platform and allowed to stay there for 15 s. The swim path and latency in finding the platform during the water maze test is analysed.

25 The hidden-platform version of the water maze task is used to assess spatial performance during the last four days of the experimental period. Animals from both control and experimental groups are subjected to the test. Both total swim path and escape latencies are analysed. The results are expected to be similar with respect to both parameters; thus, only swim paths are presented. Animals treated with AF are 30 supposed to show to perform significantly better on the spatial learning task. The analysis using one-way repeated measures with ANOVA, are expected to demonstrate a significant effect on swim distance in the AF treated group compared with the control group.

SEQUENCE LISTING

Sequence ID NO:1

5	Met Val Leu Glu Ser Thr Met Val Cys Val Asp Asn Ser
	5 10
	Glu Thr Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln
	15 20 25
10	Gln Asp Ala Val Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro
	30 35 40 45
15	Glu Asn Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu
	50 55 60
	Thr Thr Leu Thr Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr
	65 70 75
20	Val Gln Pro Lys Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala
	80 85 90
	His Leu Ala Leu Lys His Arg Gln Gly Lys Asn His Lys Met Arg Ile
	95 100 105
25	Ile Ala Phe Val Gly Ser Pro Val Glu Asp Asn Glu Lys Asp Leu Val
	110 115 120 125
30	Lys Leu Ala Lys Arg Leu Lys Lys Glu Lys Val Asn Val Asp Ile Ile
	130 135 140
	Asn Phe Gly Glu Glu Val Asn Thr Glu Lys Leu Thr Ala Phe Val
	145 150 155
35	Asn Thr Leu Asn Gly Lys Asp Gly Thr Gly Ser His Leu Val Thr Val
	160 165 170
	Pro Pro Gly Pro Ser Leu Ala Asp Ala Leu Ile Ser Ser Pro Ile Leu

CLAIMS

1. Use of a polypeptide comprising an amino acid sequence of Formula I:

5 X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent

X2 is H, R or K

10 X3 is S or L

X4 is T or A

X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent,
or a pharmaceutically acceptable salt thereof,

in the manufacture of a medicament for the treatment and/or prevention of a condition
associated with or characterised by a pathological loss and/or gain of cells.

15 2. Use as claimed in claim 1, wherein Formula I has the sequence of one of:

a) amino acids nos. 35-42 of SEQ ID NO:1

20 b) amino acids nos. 35-46 of SEQ ID NO:1

c) amino acids nos. 36-51 of SEQ ID NO:1

d) amino acids nos. 36-80 of SEQ ID NO:1

e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

25 3. Use as claimed in claim 1 or claim 2, wherein the condition is characterised by
displaying a pathological degeneration of, loss of ability and/or loss of control of
regeneration of and/or loss of control of regeneration of a differentiated cell and/or
tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived
30 from a stem cell or progenitor cell.

4. Use as claimed in any one of claims 1 to 3 wherein the condition is associated
with or characterised by a pathological loss and/or gain of cells in the peripheral
nervous system or central nervous system.

5. Use as claimed in any one of claims 1 to 4, wherein the condition is associated with or characterised by a pathological loss and/or gain of neural stem cells or neural progenitor cells.

5 6. Use as claimed in any one of claims 1 to 4, wherein the condition is associated with or characterised by a pathological loss and/or gain of oligodendroglial, astroglial, and/or neuronal cells and/or cell populations.

10 7. Use as claimed in claim 6, wherein the condition is associated with or characterised by a pathological loss and/or gain of non-cholinergic neuronal cells, cholinergic neuronal cells and/or glial cells, and/or cell populations.

8. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by damage to the central nervous system or a defect in the central nervous system.

15 9. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder.

20 10. Use as claimed in any one of claims 1 to 7, wherein the condition is caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS and/or damage to the spinal cord after disease and/or trauma.

25 11. Use as claimed in any one of claims 1 to 10, wherein said condition is characterised by memory loss.

12. Use as claimed in any one of claims 1 to 8, wherein the condition is multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.

30 13. Use of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

35 wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent
X2 is H, R or K

X3 is S or L

X4 is T or A

X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof,

5 in the manufacture of a medicament for the treatment and/or prevention of multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.

14. Use as claimed in claim 13, wherein Formula I has the sequence of one of:

10 a) amino acids nos. 35-42 of SEQ ID NO:1
 b) amino acids nos. 35-46 of SEQ ID NO:1
 c) amino acids nos. 36-51 of SEQ ID NO:1
 d) amino acids nos. 36-80 of SEQ ID NO:1

15 e) amino acids nos. 1-80 of SEQ ID NO:1,
 or a pharmaceutically acceptable salt thereof.

15. Use as claimed in any one of the previous claims, wherein the medicament is formulated for intravenous infusion, intramuscular injection and/or subcutaneous injection.

20 16. Use as claimed in any one of the previous claims, wherein the medicament is formulated so that the active substance will pass into the ventricles of a patient's brain when it is administered to said patient.

25 17. Use as claimed in any one of claims 1 to 15, wherein the medicament is formulated so that the active substance will pass into the cerebrospinal fluid of a patient when it is administered to said patient.

30 18. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer *in vitro*, characterised by treating the isolated cell with a polypeptide comprising an amino acid sequence of Formula I:

35 X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent

X2 is H, R or K

X3 is S or L

X4 is T or A

5 X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

19. A method as claimed in claim 18, wherein Formula I has the sequence of one of:

10 a) amino acids nos. 35-42 of SEQ ID NO:1

b) amino acids nos. 35-46 of SEQ ID NO:1

c) amino acids nos. 36-51 of SEQ ID NO:1

d) amino acids nos. 36-80 of SEQ ID NO:1

15 e) amino acids nos. 1-80 of SEQ ID NO:1, or a pharmaceutically acceptable salt thereof.

20. A method as claimed in claim 18 or claim 19, wherein said isolated cell is selected from the group consisting of chondrocytes, myocardial cells, blood cells, 20 neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from progenitor cells or stem cells.

21. A method of treatment and/or prevention of a condition associated with or characterised by a pathological loss and/or gain of cells, comprising administering to a 25 patient in need thereof an effective amount of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

30 wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent

X2 is H, R or K

X3 is S or L

X4 is T or A

35 X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

22. A method as claimed in claim 21, wherein Formula I has the sequence of one of:

- 5 a) amino acids nos. 35-42 of SEQ ID NO:1
- b) amino acids nos. 35-46 of SEQ ID NO:1
- c) amino acids nos. 36-51 of SEQ ID NO:1
- d) amino acids nos. 36-80 of SEQ ID NO:1
- e) amino acids nos. 1-80 of SEQ ID NO:1,

10 or a pharmaceutically acceptable salt thereof.

23. A method as claimed in claim 21 or 22, wherein the condition is characterised by displaying a pathological degeneration of, loss of ability and/or loss of control of regeneration of and/or loss of control of regeneration of a differentiated cell and/or tissue, an embryonic stem cell, an adult stem cell, a progenitor cell and/or a cell derived from a stem cell or progenitor cell.

24. A method as claimed in any one of claims 21 to 23 wherein the condition is associated with or characterised by a pathological loss and/or gain of cells in the peripheral nervous system or central nervous system.

25. A method as claimed in any one of claims 21 to 24, wherein the condition is associated with or characterised by a pathological loss and/or gain of neural stem cells or neural progenitor cells.

26. A method as claimed in any one of claims 21 to 24, wherein the condition is associated with or characterised by a pathological loss and/or gain of oligodendroglial, astroglial, and/or neuronal cells and/or cell populations.

30 27. A method as claimed in claim 26, wherein the condition is associated with or characterised by a pathological loss and/or gain of non-cholinergic neuronal cells, cholinergic neuronal cells and/or glial cells, and/or cell populations.

35 28. A method as claimed in any one of claims 21 to 27, wherein the condition is caused by damage to the central nervous system or a defect in the central nervous system.

29. A method as claimed in any one of claims 21 to 27, wherein the condition is caused by a traumatic, malignant, inflammatory, auto-immune or degenerative disorder.

30. A method as claimed in any one of claims 21 to 27, wherein the condition is
5 caused by axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS and/or damage to the spinal cord after disease and/or trauma.

31. A method as claimed in any one of claims 21 to 30, wherein said condition is
10 characterised by memory loss.

32. A method as claimed in any one of claims 21 to 28, wherein the condition is multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder.
15

33. A method of treatment and/or prevention of multiple sclerosis, asphyxia, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, Alzheimer's disease, stroke, Meniere's disease or demyelinating disorder, comprising administering
20 to a patient in need thereof an effective amount of a polypeptide comprising an amino acid sequence of Formula I:

X1-V-C-X2-X3-K-X4-R-X5 (Formula I)

25 wherein

X1 is I, amino acids nos. 1-35 of SEQ ID NO:1, or is absent

X2 is H, R or K

X3 is S or L

X4 is T or A

30 X5 is amino acids nos. 43-46, 43-51 or 43-80 of SEQ ID NO:1, or is absent, or a pharmaceutically acceptable salt thereof.

34. A method as claimed in claim 33, wherein Formula I has the sequence of one of:

35

- a) amino acids nos. 35-42 of SEQ ID NO:1
- b) amino acids nos. 35-46 of SEQ ID NO:1

- c) amino acids nos. 36-51 of SEQ ID NO:1
- d) amino acids nos. 36-80 of SEQ ID NO:1
- e) amino acids nos. 1-80 of SEQ ID NO:1,
or a pharmaceutically acceptable salt thereof.

5

35. A method as claimed in any one of claims 21 to 34, wherein the medicament is formulated for intravenous infusion, intramuscular injection and/or subcutaneous injection.

10 36. A method as claimed in any one of claims 21 to 34, wherein the medicament is formulated so that the active substance will pass into the ventricles of a patient's brain when it is administered to said patient.

15 37. A method as claimed in any one of claims 21 to 34, wherein the medicament is formulated so that the active substance will pass into the cerebrospinal fluid of a patient when it is administered to said patient.

20 38. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

25 a) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I as defined in claim 1 or claim 2 to said patient prior to isolating said cell;
b) propagating said isolated cell *in vitro*;
followed by
c) transplanting said propagated cells into the same or another patient in need thereof.

30 39. A method of propagating, inducing, reducing and/or maintaining the genesis of an isolated stem cell and/or stem cell progeny from any germinal layer from a patient, characterised by:

35 a) isolating said cell and/or stem cell progeny from the patient;
b) administering an effective amount of a polypeptide comprising the amino acid sequence of Formula I as defined in claim 1 or claim 2 to said isolated cell *in vitro* and propagating said cells;

followed by

c) transplanting said propagated cells back into the same or another patient in need thereof.

5 40. A method as claimed in claim 38 or claim 39, wherein said isolated cell is selected from the group consisting of chondrocytes, myocardial cells, blood cells, neurons, oligodendrocytes, astroglial cells, progenitor cells, stem cells and/or cells derived from progenitor cells or stem cells.

ABSTRACT**Use of anti-secretory factor peptides**

5 The invention relates to the use of certain fragments of antisecretory factor (AF) in the manufacture of a medicament for inducing proliferation, apoptosis, differentiation and/or migration of an embryonic stem cell, adult stem cell, progenitor cell and/or a cell derived from a stem cell or progenitor cell, for treating a condition characterised by or associated with loss and/or gain of cells. In a preferred embodiment, the condition is a

10 condition or disease of the CNS and/or PNS, for example, Alzheimer's disease.

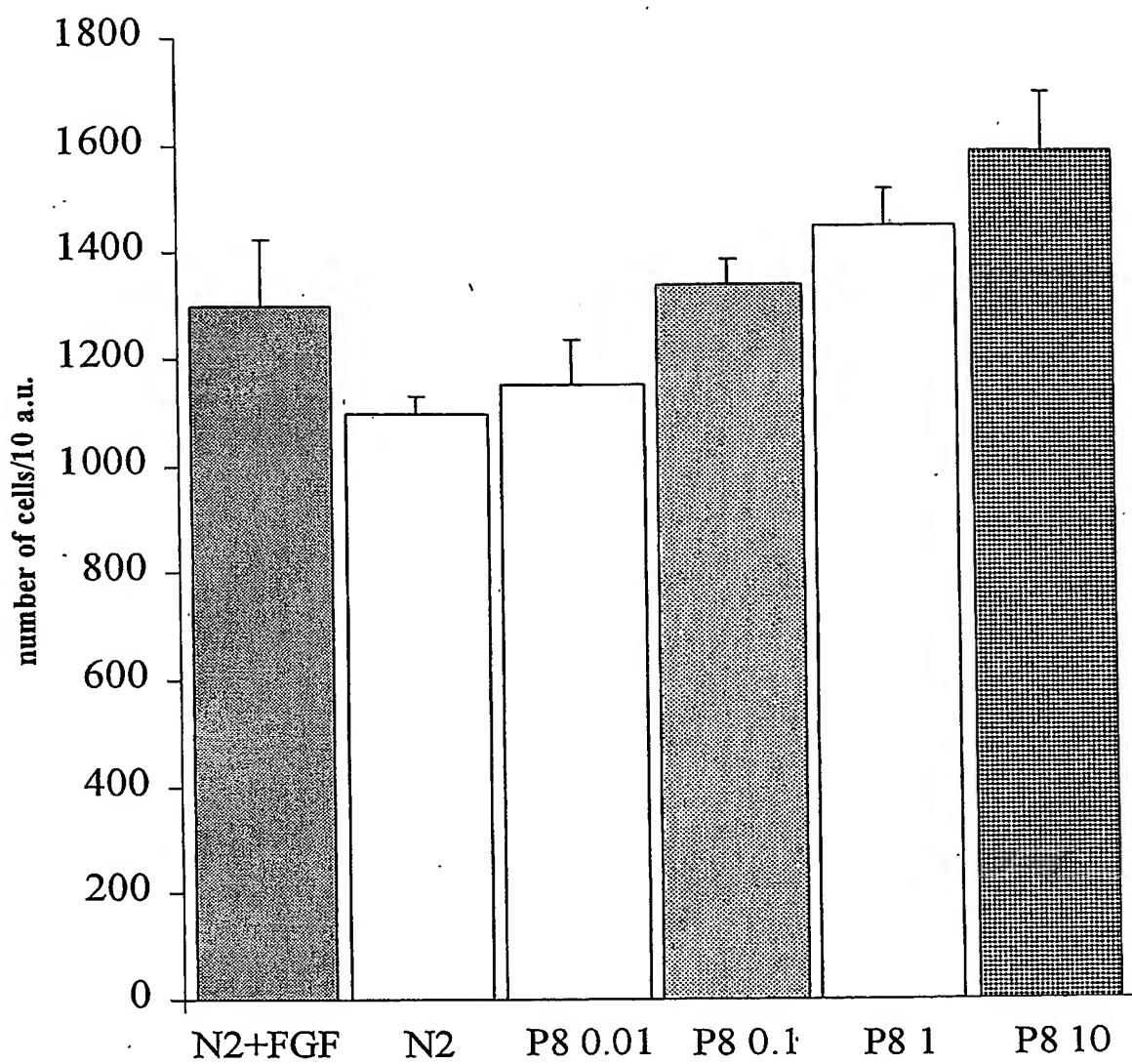
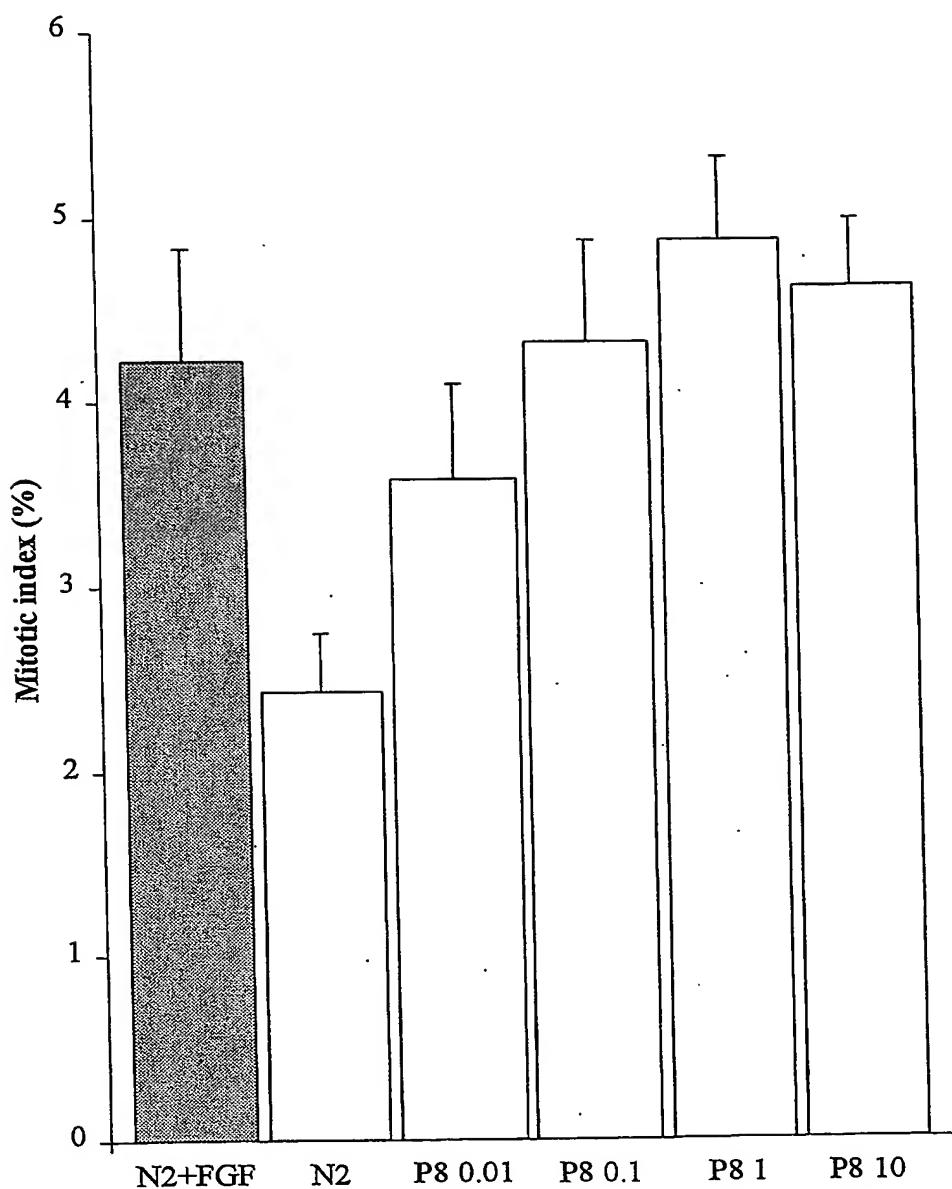
Figure 1A

Figure 1B



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10

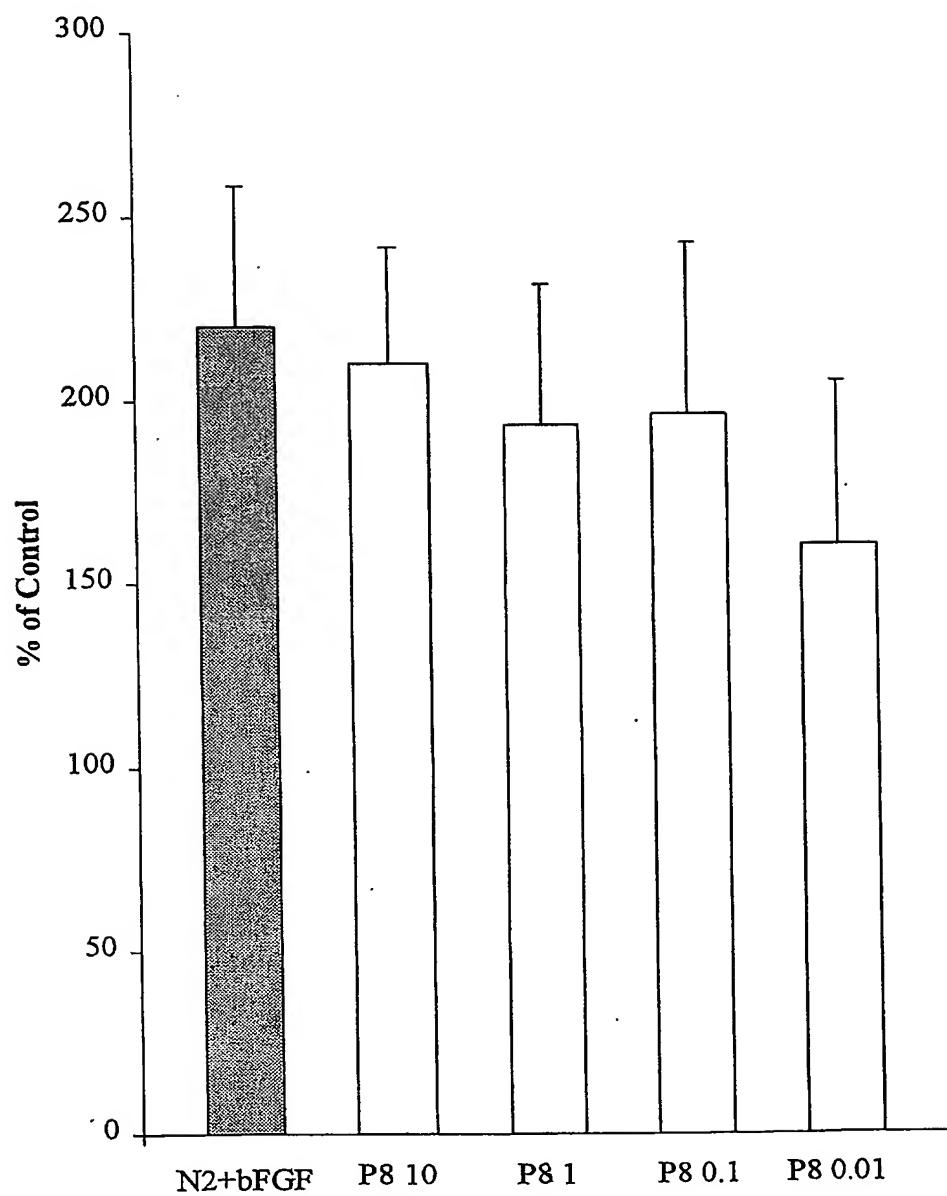
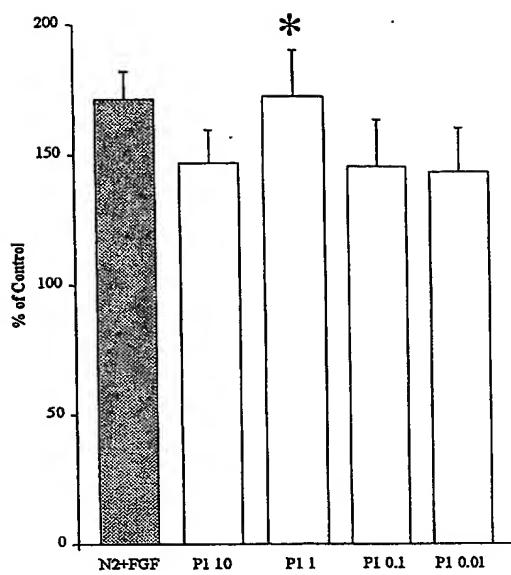
Figure 2A

Figure 2B

5

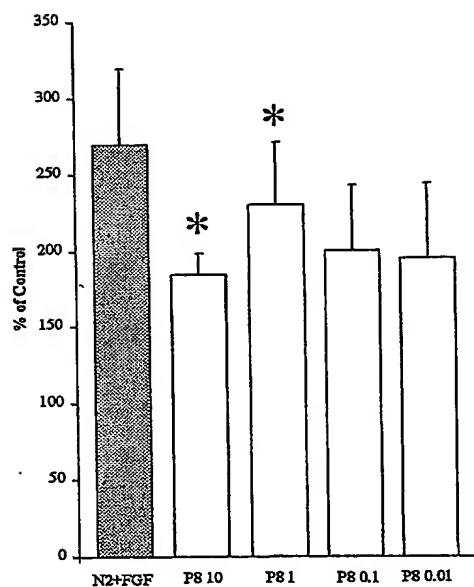


Figure 3

AATTGGAGGAGT	TGTTGTTA	GGCCGTCCCG	GAGACCCGGT	CGGGGAG												
GAAGGTGGCAAG	ATG	GTG	TTG	GAA	AGC	ACT	ATG	GTG	TGT	GTG	GAC	AAC	AGT	101		
	Met	Val	Leu	Glu	Ser	Thr	Met	Val	Cys	Val	Asp	Asn	Ser			
	5	10														
GAG	TAT	ATG	CGG	AAT	GGA	GAC	TTC	TTA	CCC	ACC	AGG	CTG	CAG	GCC	CAG	149
Glu	Thr	Met	Arg	Asn	Gly	Asp	Phe	Leu	Pro	Thr	Arg	Leu	Gln	Ala	Gln	
15	20	25														
CAG	GAT	GCT	GTC	AAC	ATA	GTG	TGT	CAT	TCA	AAG	ACC	CGC	AGC	AAC	CCT	197
Gln	Asp	Ala	Val	Asn	Ile	Val	Cys	His	Ser	Lys	Thr	Arg	Ser	Asn	Pro	
30	35	40	45													
GAG	AAC	AAC	GTG	GGC	CTT	ATC	ACA	CTG	GCT	AAT	GAC	TGT	GAA	GTG	CTG	245
Glu	Asn	Asn	Val	Gly	Leu	Ile	Thr	Leu	Ala	Asn	Asp	Cys	Glu	Val	Leu	
50	55	60														
ACC	ACA	CTC	ACC	CCA	GAC	ACT	GGC	CGT	ATC	CTG	TCC	AAG	CTA	CAT	ACT	293
Thr	Thr	Leu	Thr	Pro	Asp	Thr	Gly	Arg	Ile	Leu	Ser	Lys	Leu	His	Thr	
65	70	75														
GTC	CAA	CCC	AAG	GGC	AAG	ATC	ACC	TTC	TGC	ACG	GGC	ATC	CGC	GTG	GCC	341
Val	Gln	Pro	Lys	Gly	Lys	Ile	Thr	Phe	Cys	Thr	Gly	Ile	Arg	Val	Ala	
80	85	90														
CAT	CTG	GCT	CTG	AAG	CAC	CGA	CAA	GGC	AAG	AAT	CAC	AAG	ATG	CGC	ATC	389
His	Leu	Ala	Leu	Lys	His	Arg	Gln	Gly	Lys	Asn	His	Lys	Met	Arg	Ile	
95	100	105														
ATT	GCC	TTT	GTG	GGA	AGC	CCA	GTG	GAG	GAC	AAT	GAG	AAG	GAT	CTG	GTG	437
Ile	Ala	Phe	Val	Gly	Ser	Pro	Val	Glu	Asp	Asn	Glu	Lys	Asp	Leu	Val	
110	115	120	125													
AAA	CTG	GCT	AAA	CGC	CTC	AAG	AAG	GAG	AAA	GTA	AAT	GTT	GAC	ATT	ATC	485
Lys	Leu	Ala	Lys	Arg	Leu	Lys	Glu	Lys	Lys	Val	Asn	Val	Asp	Ile	Ile	
130	135	140														
AAT	TTT	GGG	GAA	GAG	GAG	GTG	AAC	ACA	GAA	AAG	CTG	ACA	GCC	TTT	GTA	533
Asn	Phe	Gly	Glu	Glu	Glu	Val	Asn	Thr	Glu	Lys	Leu	Thr	Ala	Phe	Val	
145	150	155														
AAC	ACG	TTG	AAT	GGC	AAA	GAT	GGA	ACC	GGT	TCT	CAT	CTG	GTG	ACA	GTG	581
Asn	Thr	Leu	Asn	Gly	Lys	Asp	Gly	Thr	Gly	Ser	His	Leu	Val	Thr	Val	
160	165	170														
CCT	CCT	GGG	CCC	AGT	TTG	GCT	GAT	GCT	CTC	ATC	AGT	TCT	CCG	ATT	TTG	629
Pro	Pro	Gly	Pro	Ser	Leu	Ala	Asp	Ala	Leu	Ile	Ser	Ser	Pro	Ile	Leu	
175	180	185														
GCT	GGT	GAA	GGT	GGT	GCC	ATG	CTG	GGT	CTT	GGT	GCC	AGT	GAC	TTT	GAA	677
TTT	GGA	GTA	GAT	CCC	AGT	GCT	GAT	CCT	GAG	CTG	GCC	TTG	GCC	CTT	CGT	725
GTA	TCT	ATG	GAA	GAG	CAG	CGG	CAC	GCA	GGA	GGA	GGA	GCG	CGG	CGG	GCA	733
GCT	CGA	GCT	TCT	GCT	GCT	GAG	GCC	GGG	ATT	GCT	ACG	ACT	GGG	ACT	GAA	821
GAC	TCA	GAC	GAT	GCC	CTG	CTG	AAG	ATG	ACC	ATC	AGC	CAG	CAA	GAG	TTT	869
GGC	CGC	ACT	GGG	CTT	CCT	GAC	CTA	AGC	AGT	AGT	ACT	GAG	GAA	GAG	GAG	917

ATT GCT TAT GCC ATG CAG ATG TCC CTG CAG GGA GCA GAG TTT GGC CAG 965
GCG GAA TCA GCA GAC ATT GAT GCC AGC TCA GCT ATG GAC ACA TCT GAG 1013
CCA GCC AAG GAG GAG GAT GAT TAC GAC GTG ATG CAG GAC CCC GAG TTC 1061
CTT CAG AGT GTC CTA GAG AAC CTC CCA GGT GTG GAT CCC AAC AAT GAA 1109
GCC ATT CGA AAT GCT ATG GGC TCC CTG CCT CCC AGG CCA CCA AGG ACG 1157
GCA AGA AGG ACA AGA AGG AGG AAG ACA AGA AGT GAG ACT GGA GGG AAA 1205
GGG TAGC TGAG TCTG CTTA GGGG ACTG GGAA GCAC GGAA TATA GGGT TAGA
TGTG GTTA TCTG TAAC CATT ACAG CCTA AATA AAGC TTGG CAAC TTTT AAAA
AAAA AAAA AAAA AAA

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